PATENT
Attorney Docket No.07681.0018-01
(710/205C)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
)
CAMPBELL et al.) Group Art Unit: 1632
)
Serial No.: TO BE ASSIGNED) Examiner: D. Crouch
)
Filed: November 21, 2001)
)
For: UNACTIVATED OOCYTES AS)
CYTOPLAST RECIPIENTS)
FOR NUCLEAR TRANSFER)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

SUPPORT FOR APPLICANTS' CLAIM 19

Limitations in Applicants' claim 19	**Support in Application**
19. A method of producing	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Page 1, lines 4-7). . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Page 6, lines 9-12). 13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Page 30, lines 17-25).

Limitations in Applicants' claim 19	Support in Application
a non-human mammalian embryo	Since the demonstration of embryo cloning in amphibians, similar techniques have been applied to mammalian species. These techniques fall into two categories: 1) transfer of a donor nucleus to a matured metaphase II oocyte which has had its chromosomal DNA removed and 2) transfer of a donor nucleus to a fertilised one cell zygote which has had both pronuclei removed. In ungulates the former procedure has become the method of choice as no development has been reported using the latter other than when pronuclei are exchanged. . . . equivalent totipotent nuclei from a single individual could, when transferred to an enucleated egg, give rise to "genetically identical" individuals. (Page 1, line 32 - page 2, line 1). In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).
by nuclear transfer comprising:	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient

Limitations in Applicants' claim 19	Support in Application
	oocyte. (Page 7, lines 16-18). Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29).
(i) transfer of a nucleus	Once suitable donor and recipient cells have been prepared, it is necessary for the nucleus of the former to be transferred to the latter. (Page 10, lines 26-28).
of a non-human mammalian cell,	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).
which has passed start in the mitotic cell cycle and is in the G1 phase of the cell cycle,	In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24). The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32). . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 21-24).
into an unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce

Limitations in Applicants' claim 19	Support in Application
	oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21). In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8).
enucleated,	It is preferred that the recipient be enucleated (Page 9, line 6).
metaphase II-arrested oocyte	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31). In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28).

Limitations in Applicants' claim 19	Support in Application
	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. (Page 5, lines 1-12).
of the same species as the donor cell nucleus;	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31).
(ii) activation of the recipient oocyte containing the donor cell nucleus; and	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6). The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32).
(iii) incubation of the oocyte to provide an embryo;	Aside from the issue of yield-improving expediencies, the reconstituted embryo may be cultured, *in vivo* and *in vitro* to blastocyst. (Page 17, line 1-3). The process can be regarded as involving five steps: * * * (4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, lines 14-24).
wherein the donor cell nucleus is from a mammalian differentiated cell.	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can

Limitations in Applicants' claim 19	Support in Application
	cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19).

236659